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Defining a research agenda to address the converging epidemics of tuberculosis and diabetes. Part 2: Underlying biological mechanisms

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Abstract

There is growing interest in the re-emerging interaction between type 2 diabetes (DM) and tuberculosis (TB), but the underlying biological mechanisms are poorly understood despite their possible implications in clinical management. Experts in epidemiological, public health, basic science and clinical studies recently convened and identified research priorities for elucidating the underlying mechanisms for the co-ocurrence of TB and DM. We identified gaps in current knowlege of altered immunity in DM patients during TB, where most studies suggest an under-performing innate immunity, but exaggerated adaptive immunity to *Mycobacterium tuberculosis*. Various molecular mechanisms and pathways that may underly these observations in the DM host. These include signaling induced by excess advanced glycation end products (AGE) and their receptor (RAGE), higher levels of reactive oxidative species and oxidative stress, epigenetic changes due to chronic hyperglycemia, altered nuclear receptors and/or differences in cell metabolism (immuno-metabolism). Studies in humans at different stages of DM (no DM, pre-DM and DM) or TB (latent or active TB) should be complemented with findings in animal models, which provide the unique opportunity to study early events in the host-pathogen interaction. Such studies could also help identify biomarkers that will complement clinical studies in order to tailor the prevention of TB-DM, or avoid the adverse TB treatment outcomes that are more likely in these patients. Such studies will also inform new approaches to host-directed therapies.

Summary box

- **Type 2 diabetes is a syndrome characterised by a range of metabolic (e.g. hyperglycemia, hyperlipidemia), inflammatory, vascular and other changes that may all contribute to increasing TB susceptibility and pathology.**
- **Monocytes and macrophages from diabetic patients and mice have defects leading to altered interactions with *M. tuberculosis* and delayed adaptive immune responses.**
- **Most human studies have been conducted in patients with active TB, where those with TB-DM comorbidity are characterised by increased secretion of Th1, Th17 and Th2 cytokines. However, the few studies in individuals at risk for TB (LTBI) suggest a different cytokine profile.**
- **Molecular pathways that involve AGE/RAGE, ROS, nuclear receptors and cellular metabolism are potential targets for host-directed therapies to reduce TB susceptibility or pathology in DM patients.**
- **Animal models for TB-DM can improve our understanding of underlying mechanisms and effective treatment approaches for the comorbidity of TB and DM.**

Introduction

Type 2 diabetes mellitus (DM) increases the risk of many infectious diseases, including tuberculosis[1] (TB), and it is now recognized that the increasing DM prevalence in high TB incidence countries such as Sub-Saharan Africa (SSA) is a challenge to TB control.[2] The known association between a chronic syndome like DM and an infectious disease like TB requires the near term development of a comprehensive research agenda that effectively integrates the basic sciences with clinical decision making and policy to reduce the impact of the co-morbidity. To address this research agenda, a group of international TB and DM experts convened at the National Institutes of Health (NIH) in May 2016 to discuss the convergent epidemics of DM and TB along with HIV. In this Part 2, we summarize the biological mechanisms that were identified as research priorities.

Knowledge of the altered biological mechanisms and pathways associated with TB and DM is needed to help identify the subgroup of DM patients at highest risk of progression to TB. This knowledge will also benefit DM patients with a new diagnosis of TB will require modifications in the standard TB treatment schedule in order to prevent adverse treatment outcomes. It is increasingly clear that although TB and DM have different pathogenic mechanisms, they also share a number of similarities at the molecular level, including key pathways involved in chronic inflammation, metabolism and immunity.[3-5] It is critical to gain insight into the factors underlying the links between TB and DM at the molecular, cellular and systemic levels and to integrate data from clinical studies and animal models to better understand the fundamental causes and consequences of the comorbidity.

Altered immunity: The effect of pre-DM and DM on human immunity to M. tuberculosis during TB and LTBI

Recent reviews have addressed the effect of DM on host response to *M. tuberculosis*[4,6,7]. Studies on human innate immune responses indicate that monocytes from poorly-controlled DM patients (versus well-controlled or non-DM) have significantly lower binding

and phagocytosis of *M. tuberculosis*, and this defect is attributable to alterations in the diabetic monocyte as well as in serum opsonins[8-10]. Efficient phagocytosis and proper adaptive immune priming are necessary to activate cell-mediated immune responses that restrict *M. tuberculosis* growth, and delayed or altered responses likely contribute to diabetic TB susceptiblity[11]. Diabetic individuals with LTBI have lower frequencies of *M. tuberculosis*-specific pro-inflammatory (Th1 and Th17), anti-inflammatory (IL-10) and Th2 responses compared to normoglycemic individuals with LTBI[12,13]. The IL-20 family of cytokines is also lower in LTBI-DM whereas IL-22 is higher[14]. Once patients have developed active TB disease, those with DM exhibit higher circulating levels of Th1 and Th17 (except for IL-22) cytokines as well as higher frequencies of lymphocytes (CD4+, CD8+) and NK cells expressing these cytokines in response to *M. tuberculosis* antigens[15-19]. However, TB-DM patients also have higher levels of anti-inflammatory cytokines, notably IL-10[16]. The higher expression of pro-inflammatory cytokines could reflect higher bacillary load in TB-DM, as a consequence of a delayed initial control of *M. tuberculosis* replication (along with increased tissue damage) as a consequence of weak cytokine responses to LTBI in humans. Other contributing factors may be a reduced frequency of natural regulatory T cells in TB-DM patients[17] and hyper-responsiveness to T cell antigen receptor stimulation as identified in diabetic mice[20]. Nearly all studies on innate and adaptive immune responses in TB-naive, LTBI and TB patients have been conducted in peripheral blood cells. However, one study in TB-DM patients evaluated the lung environment, and showed higher IL-10 and lower IFN-γ in TB-DM, suggesting an anti-inflammatory bias in this compartment as well[21].

Few studies have focused on individuals with pre-DM (characterized by insulin resistance and pancreatic β-cell dysfunction prior to detectable changes in glycemic control) or intermediate hyperglycemia despite their high risk for future DM. Babu et al[12,22] have focused on investigating the influence of pre-DM on antigen-stimulated cytokine production in active TB and LTBI. Individuals with pre-DM and active TB have increased circulating levels of Th1 (IFNγ, TNFα, IL2), Th2 (IL-4, IL-5), Th17 (IL-17A, IL-17F) and regulatory cytokines (IL-10, TGFβ) compared to TB patients without pre-DM. However, IL-22 concentrations do not differ. Individuals with LTBI and pre-DM exhibited diminished circulating levels of Th1, Th2, Th17 and regulatory cyokines compared to normoglycemic participants with LTBI, as well as decreased *M. tuberculosis* antigen-stimulated cytokine concentrations.

Together, studies on human immunity in TB-naive, LTBI or TB patients indicate dysfunctional immunity in pre-DM and DM patients that calls for further studies. The mechanisms and impact of the defects observed on *M. tuberculosis* growth containment as well as immune pathology are incompletely understood. Furthermore, there is a paucity of studies evaluating the lung, which is the primary site of TB disease, and the relationship between the immune responses to *M. tuberculosis* in the lung and periphery is poorly understood. Given the higher prevalence of pulmonary (versus extrapulmonary) TB among DM patients,understanding this compartmentalization is particularly relevant[4,23]. Data from the mouse TB-DM model suggest that chronic hyperglycemia exerts unique effects on alveolar versus peritonal and bone marrow-derived macrophages[24]. Thus, integration of the observed immunometabolic abnormalities in pre-diabetic and diabetic hosts warrant futher investigation.

Advanced glycation end products and RAGE signaling

Advanced glycation end products (AGEs) accumulate during metabolic disorders fueled by hyperglycaemia. The receptor for AGEs, RAGE, is expressed on a variety of cell types including those highly relevant in the context of TB and DM (e.g., monocytes and macrophages, dendritic cells, T-cells and vascular cells). Interestingly, the highest expression of RAGE occurs in the lungs[25], the primary site of *M. tuberculosis* infection. Activation of RAGE up-regulates inflammation through the production of reactive oxigen species (ROS) and inflammatory cytokines, and alters phagocytosis and cellular lipid metabolism.

At the present time, there are no approved drugs that target AGEs or RAGE in the treatment of DM and diabetic complications. However, a new class of 2-aminoimidazole-based small molecules have been shown to have potent anti-AGE activity in vitro[26]. Inhibition or blocking the pro-inflammatory response as a consequence of AGE-RAGE interactions may prove to be an effective adjunctive therapy in the treatment of TB-DM comorbity.

ROS as a central mediator

In the host defence against mycobacteria, ROS regulates cytokine production, autophagy and granuloma formation[27], but excessive ROS production leads to impaired cellular function and pathology. Hyperglycemia- and free fatty acid-induced overproduction of ROS activates the major pathways of diabetic cellular damage. Furthermore, hyperglycemia-induced ROS production leads to histone modifications in the NFkB p65 proximal promoter resulting in gene activation of this major regulator of inflammatory genes[28]. Although increased mitochondrial ROS production enhances mycobacterial killing in macrophages, increased ROS production can also increase necroptosis and mycobacterial release into the extracellular milieu[29,30]. Therefore, DM metabolite-induced increased ROS production may further contribute to the increased rate of relapse and death in TB patients with poor glycemic control. The diabetic phenotypes of alveolar macrophage recognition of *M. tuberculosis* and T cell hyper-responsiveness were also shown to be at least partially dependent on RAGE expression[57, 24]. Thus, blocking the RAGE signaling pathway may reduce ROS generation and may prove useful in the context of TB-DM comorbidity.

Nuclear receptors

Another potential family of therapeutic targets and key molecular players in metabolic and immunological pathways are nuclear receptors (NRs). Peroxisome proliferator-activated receptors (PPARs) are highly expressed in a variety of tissues including adipose tissue, macrophages and dendritic cells and play a major role in lipid metabolism, but also in innate and adaptive immunity. PPARγ is of particular interest as it is also highly expressed in alveolar macrophages, where it contributes to the formation of foam cells and promotes anti-inflammatory gene expression while transrepressing pro-inflammatory gene expression upon ligand binding[31]; it also serves as a biological marker for alternatively activated macrophages (M2 phenotype)[32]. *M. tuberculosis* infection of macrophages induces PPARγ via the mannose receptor (MR, CD206)[33] and TLR2[34] and in turn increases *M. tuberculosis* intracellular growth, lipid body formation and chemokine release. PPARγ knock down followed by *M. tuberculosis* infection leads to decreased growth, and an increase in expression of 36 genes (including BAX) and a decrease in expression of 31 genes. Therefore, it is possible that activation of PPARγ by *M. tuberculosis* limits cellular apoptosis by inhibiting BAX expression and inducing Mcl-1 (Arnett and Schlesinger, unpublished). One

could envision that PPARγ antagonists could potentially be used to prevent primary TB infection, whereas PPARγ agonists (which are used in the treatment of DM) could have a beneficial effect as an adjunct host-directed therapy to reduce inflammation during active TB disease.

Altered host metabolism in tuberculosis

It has recently been shown that *M. tuberculosis* induces a switch in host cellular metabolism towards aerobic glycolysis in humans. The metabolic switch is TLR2-dependent but NOD2-independent, and is mediated in part through activation of the AKT-mTOR pathway[35]. Pharmacological inhibition of the AKT/mTOR pathway inhibits cellular responses to *M. tuberculosis* both in vitro and in vivo in a model of murine TB. Another study showed how responses to BCG depend on changes in cellular metabolism and epigenetics.[36] These findings reveal a novel regulatory layer of host responses to *M. tuberculosis* that could be exploited for host-directed therapy. Indeed, the antidiabetic drug metformin, which inhibits mTOR through induction of AMPK (adenosine monophosphate–activated protein kinase), was shown to increase mitochondrial reactive oxygen species, facilitate phagosome-lysosome fusion and reduce growth of *M. tuberculosis* in macrophages.[37] In this same study, metformin ameliorated lung pathology, reduced chronic inflammation, and enhanced the specific immune response and efficacy of conventional TB drugs in *M. tuberculosis*-infected mice. Similarly, metformin treatment in the guinea pig model of TB restored systemic glucose metabolism and lessened pulmonary pathology (Basaraba and Podell unpublished). This work should be extended, also evaluating effects of other antidiabetic drugs, to establish the role of cellular metabolism in TB-DM.

Mouse models to study TB-DM comorbidity

In TB-DM mouse models, susceptibility to TB is observed with chronic but not acute hyperglycemia.[38,39] Chronic hyperglycemia in mice impairs the innate response of resident alveolar macrophages to inhaled *M. tuberculosis*. The resulting delay in recruiting myeloid cells, including neutrophils and dendritic cells to the alveolar airspace, leads to a delay in transferring bacilli from the lung to the lymph node and a delay in priming the adaptive immune response[40]. Alveolar macrophages from diabetic mice have reduced CD14 and macrophage receptor with collagenous structure (MARCO) expression and display reduced phagocytosis[24]. Transfer of infected alveolar macrophages from diabetic mice into normoglycemic recipients confirmed an intrinsic defect that hinders T cell priming. This delay permits several additional days of logarithmic increase in lung bacterial load before antigen-specific T cells reach the lung and restrict bacterial replication. The phenotype of diabetic alveolar macrophages is not shared by macrophages from other compartments in diabetic mice, such as the peritoneal or bone marrow-derived macrophages of chronic hyperglycemic mice. This unique macrophage phenotype appears to be dependent in part on the expression of RAGE[24]. Once the immune response to *M. tuberculosis* is initiated in the DM mice, it is excessive. In a recent study, the interaction between NK and CD11c+ (dendritic cells) led to excessive IL-6 driven immune pathology in DM mice.[39] Naive T cells in diabetic mice display chromatin decondensation similar to activated T cells. This chromatin decondensation is also RAGE dependent and persists upon adoptive transfer to a non-diabetic host manifesting in increased expression of a broad range of cytokines and

increased proliferation of stimulated diabetic versus normoglycemic T cells[20]. Similar to diabetic mice, DM patients show increased immune pathology and increased expression of a broad range of Th1, Th2 and Th17 cytokines that could not otherwise be attributed to a perturbation of signal transduction through any one particular pathway.

Overall, the mouse offers an informative approach to model the mechanisms of TB susceptibility in humans with DM. Furthermore, the alveolar macrophage phenotype of mice suggests that a major adverse effect of DM occurs months prior to the ususal timing of clinical TB diagnosis and might be mediated by epigenetic programming.

Guinea pig models for DM and TB

The guinea pig displays a similar pathology and metabolic response to *M. tuberculosis* infection as seen in humans. The guinea pig model utilized in co-morbidity studies by Podell and collegues[41] closely replicates the pathogenesis of human type 2 DM, which is important since dyslipidemia, hyperinsulinemia and insulin resistance are all potential contributing factors in human diabetic immunopathy. Like diabetic guinea pigs, pre-diabetic guinea pigs had a higher pulmonary and extra-pulmonary bacterial burden and an increased expression of pro-inflammatory cytokines in the late stages of infection compared to non-diabetic animals. Compared to normal guinea pigs, IFN-γ, IL-17, TNF-α and IL-1β levels were elevated in the spleen. At day 30 post-infection in diabetic guinea pigs, the high lung and extra-pulmonary *M. tuberculosis* burden was accompanied by a neutrophil-driven inflammatory response resulting in more severe granuloma necrosis. Despite elevated Th1 responses, guinea pigs were unable to contain bacterial growth and they display exacerbated immunopathology. Also similar to the mouse model, the delivery of viable bacteria to the lung-draining lymph nodes was delayed in guinea pigs due to a cellular defect where antigen-presenting cells in DM remain in a state of immaturity and have impaired capacity to migrate towards chemoattractant stimuli (Podell et al. unpublished). Diabetic guinea pigs had higher mortality during TB treatment than non-diabetic control animals or those with diet-induced impaired glucose tolerance, which corresponds to the increased TB mortality in human patients with TB-DM comorbidity. Taken together, the guinea pig model complements the mouse model and shares important similarities with the naturally occuring disease in humans and is an essential tool to better understand the underlying mechanisms of TB-DM comorbidity, as well as interrogate new therapeutic and preventative therapies.

Future perspectives and research prioirities

TB and DM have a complex interaction affecting a number of molecular pathways that we are just beginning to be understand. Knowledge of these pathways will directly impact the approaches we take to diagnosis, treatment and prevention. During the expert meeting some important conclusions and priority areas for further study were identified.

First, *different mechanisms may underly increases in TB susceptibility, early deaths, disease severity and TB recurrence in DM patients.* It will be necessary to tease out specific epidemiological links and do careful phenotyping to select the most appropriate individuals for basic science studies. Such studies could also help identify biomarkers to direct treatment and foster basic research of the interaction of TB and DM. Phase II clinical trials should examine possible host-directed strategies.

Basic research should focus on immune-metabolic pathways and other molecular mechansims underlying defective anti-mycobacterial immune responses in DM, capitalizing on knowledge gained in the cancer field relating to drugs, drug targets and host signaling pathways that impact immunology and metabolism. The impact of DM on memory T cell expansion and lifespan is unexplored, as are the potential effects of DM on T cell senescence. Likewise, the mechanisms underlying aberrant pro-inflammatory signaling pathways in the innate immune system in DM need further exploration since they likely directly influence alterations in the *developing adaptive immune response.*

Animal models offer opportunities to investigate early events in the host-pathogen interaction relevant to human TB-DM comorbidity that are not ammenable to clinical studies; mechanisms of TB-associated metaflammation in adipose tissue; and cost-effective models for preclinical studies of host-directed therapies.

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